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# FINAL REPORT

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## "Estivation of anopheles gambiar: Potential habitats and physiology"

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## 3. Executive Summary

Over 300 million people are infected with malaria and 2-3 million African children die of the disease every year. In parts of sub-Saharan Africa, active, adult malaria vectors belonging to the *Anopheles gambiae* species group, disappear during the dry season. The speeds with which biting females reappear as soon as rains have resumed, and the almost immediate resurgence of malaria, indicate that aestivating females remain alive throughout the dry months.

Our research sought to locate aestivating mosquito populations and evaluate their potential role in recolonization during subsequent rainy seasons. In Mali, a thorough field study of potential aestivation shelters was carried out. Oxygen and carbon dioxide levels as well as temperatures inside shelters were measured and exhaustive attempts were made to trap mosquitoes. In Israel, a setup for monitoring rates of respiration in small groups of mosquitoes was devised . The effects of age and malaria infections on metabolic rates were studied.

Our comprehensive efforts failed to reveal large concentrations of aestivating *An. gambiae*. Contrary to our working hypothesis, it seems that only small numbers of mosquitoes aestivate in any one shelter and that many different shelters give rise to the mosquitoes of the early rainy season. Hence, although the dry season constitutes a population bottle –neck, the aestivating mosquitoes are dispersed and largely inaccessible to human intervention or manipulation.

Important findings were made with respect to the respiratory physiology of mosquitoes and the effect of age and malaria infections on metabolism (Bhasin Ar & Warburg, in preparation). Malaria infected mosquitoes require more oxygen and emit more $CO_2$ than uninfected ones.

The project teamed Dr Warburg's expertise in medical entomology with Prof Ar, an expert in respiratory physiology and Prof Toure an expert on the *An. gambiae* species complex. Malian and Israeli scientists working together, conducted extensive field studies in two villages in Mali and developed an open-flow system for monitoring metabolism of small groups of mosquitoes. Substantial scientific data was collected and scientific insights were made despite the fact that large concentrations of aestivating *An. gambiae* were not found. Capacity strengthening manifested itself in the application of new lines of thought as well as innovative research approaches toward the solution of problems unique to Africa. Three scientific publications resulting from this project, are under preparation at present.

## 4. Research Objectives

To identify potential aestivation habitats for *Anopheles gambiae*, evaluate their importance as sources for rainy season "recolonization" and the subsequent resumption of malaria transmission. Results of field studies will also clarify possible aestivation by other important malaria vectors, most notably *An. funestus*.

The following subsidiary objectives were undertaken to achieve this:

1. Devise methods for capturing mosquitoes entering or exiting potential aestivation shelters. Devise methods for extracting aestivating mosquitoes from different types of shelters. Measure the gas composition and temperature inside rodent burrows and other aestivation sites of *An. gambiae*.

2. Characterize populations of aestivating mosquitoes or those attempting to enter or exit shelters during different times of the year in terms of their karyotype, gonotrophic status and *Plasmodium* sporozoite infection rates.

3. Simulate in the laboratory, putative environmental cues for aestivation and study their effects on the physiology of mosquitoes (metabolic rate, gonotrophic cycles, longevity).

## 5. Methods and Results

### 5.1. Field Work in Mali

A number of sampling techniques were employed in the field focusing on the transition period from dry season to wet (May-Aug). We hypothesized that a suitable aestivation site would contain large numbers of aestivating *An. gambiae* s.l. To identify such sites, we sampled rodent burrows, caves, tree holes , wells and caves routinely throughout the dry seasons (Holstein 1954). Great emphasis was placed on molecular (PCR) and cytotaxonomic identification of the mosquitoes sampled during the study so that results could be correctly interpreted. The establishment of a PCR-RFLP diagnostic test in Mali allows us, for the first time, to distinguish the Mopti chromosomal form from Bamako/Savanna forms (Toure et al. 1983; Sangare et al. 1994).

Mosquitoes were collected using Pyrethroid spray catches (PSC) within houses and other peridomestic sites such as abandoned houses, animal shelters and wells. The ovaries of all female *An.gambiae* s.l. were examined to monitor ovarian and follicular development and assess the degree of fat body hypertrophy. The accumulation of metabolic reserves in the fat body has previously been suggested to be one method by which mosquitoes might survive extended periods under adverse conditions (Denlinger 1986).

A Mark-Release-Recapture experiment was initiated in September 1999 and followed through to its conclusion in June 2000. Monthly PSC catches after this date were also scrutinized for the presence of marked mosquitoes.

PCR-RFLP diagnostic test was established in the laboratory in Mali allowing the Mopti chromosomal forms to be distinguished from Bamako/Savanna. Weekly samples of *Anopheles* spp. larvae obtained from Doneguebougou and Banambani were identified using PCR. Great efforts were also made to collate the vast amount of data already gathered into a single database which could be readily accessed and used by all partners and would serve in the preparation of future publications. This work is ongoing at present (September 2002).

#### 5.1.1. Summary of Results Mali

**PSC sampling in Human dwellings** - was performed in 4 villages (Kalaban Coro, Moribabougou, Banambani and Donegubougou). Because some much data was accrued, priority was given to the identification of specimens from Doneguebougou. The results, of cytotaxonomic identification of semi-gravid females and PCR identification of other specimens are shown in Figures 1 & 2, respectively. Bars

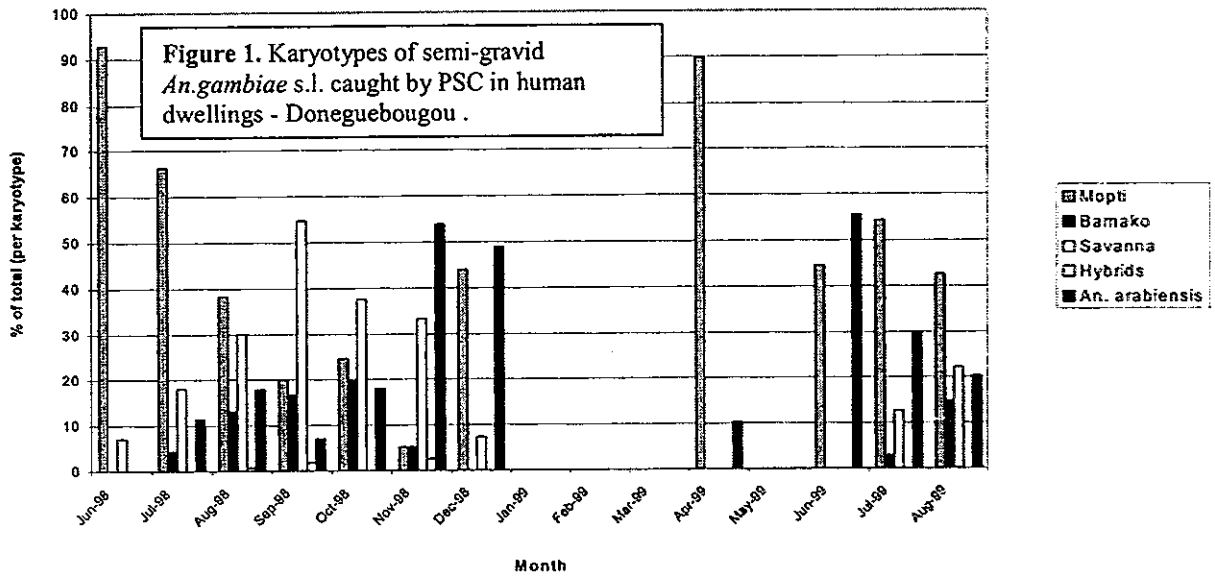
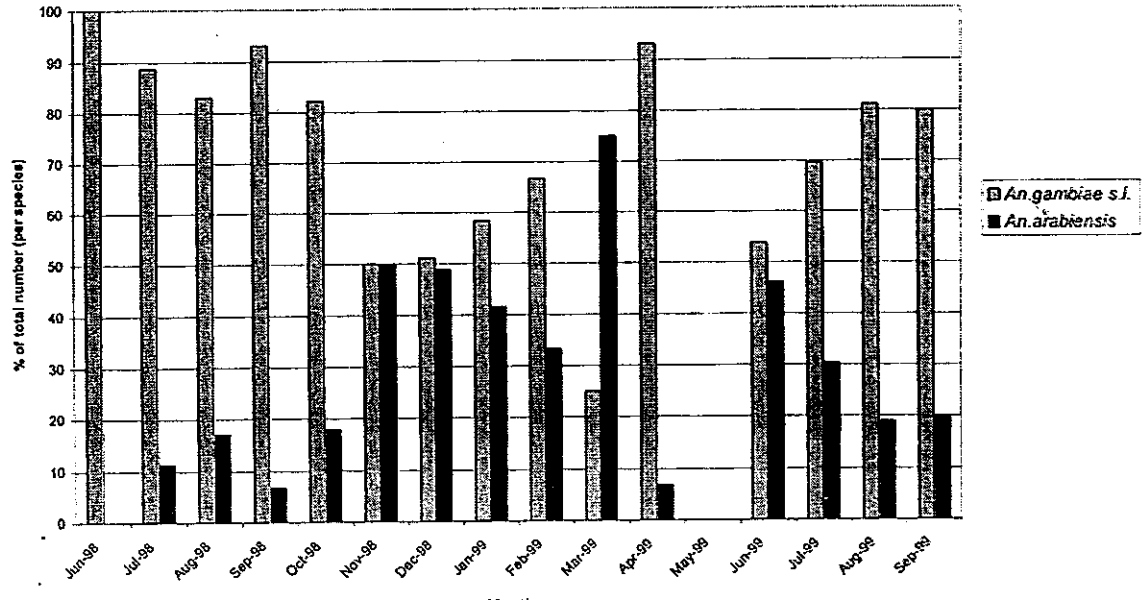



**Figure 1.** Karyotypes of semi-gravid *An.gambiae* s.l. caught by PSC in human dwellings - Doneguebougou .

indicate the monthly sample of a specific karyotype/species expressed as a proportion of the total catch.

**Figure 2.** PCR based species determination of *An.gambiae* s.l. specimens caught by PSC in Doneguebougou .



**Other Peridomestic sites** - PSC sampling of sites such as abandoned houses, chicken coops, tree holes/termite mound, all within the confines of the village, continued in parallel with that of inhabited human dwellings (Fig 3).

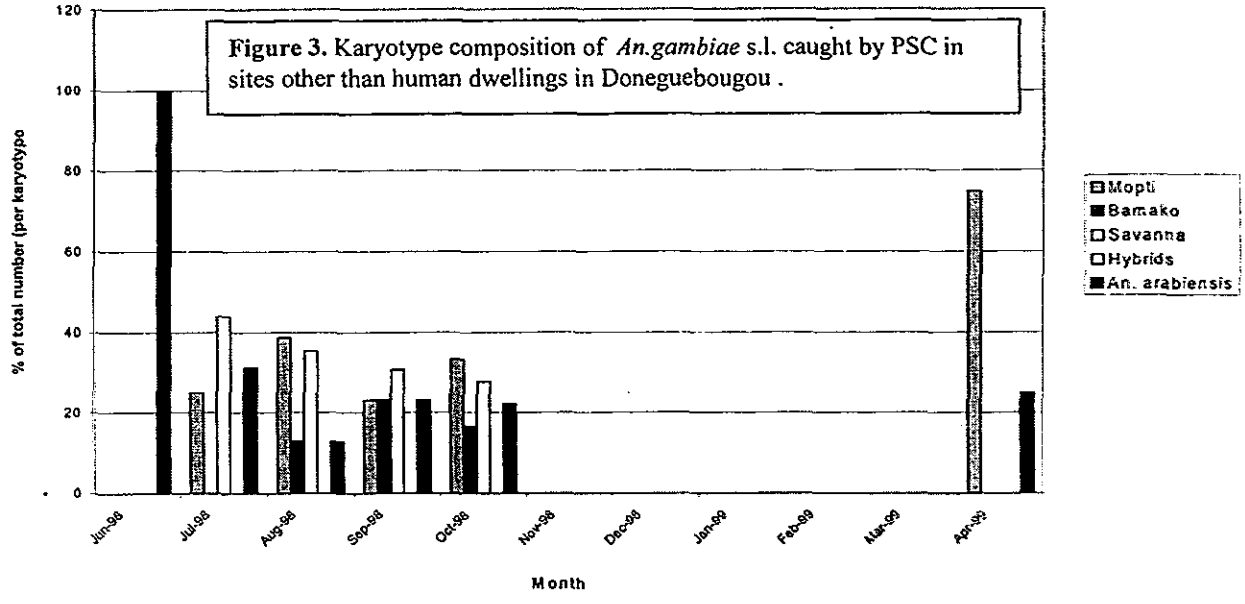



**Figure 3.** Karyotype composition of *An.gambiae* s.l. caught by PSC in sites other than human dwellings in Doneguebougou .

**Sampling of potential aestivation sites in the field.**

i) **Termite mounds.** Termite mounds offer a suitable environment for a variety on insects that have "learned" to live with the termites and enjoy their unique air conditioned environment. Active termite mounds ranging in size from 3x2m (length x width) to 5x4.5m were selected. Cotton mesh emergence cages, 1.75m high, were constructed around the termite mounds using bamboo supports. A 1m zipper incorporated into one corner of each net allowed entry of collectors into the cage. Two black cotton squares (0.5x0.5m) sewn into the eastern facing corners of each cage provided an area of shade, reducing the exposure of any insects, which may have emerged to adverse morning conditions. The mounds were monitored each morning between 0600 – 0700h and any mosquitoes within were removed with a mouth aspirator. No *An.gambiae* s.l. was collected from enclosed termite mounds despite repeated monitoring of at least 5 mounds per dry season in different villages.

ii) **Rodent burrows.** Rodent burrows frequently harbor other species both parasitic and free living (Arieli 1979; Storey & Storey 1990). Inverted funnel fitted with mosquito cages were placed over the entrances to rodent burrows and served as exit traps. 25 exit traps were placed over rodent burrows in three areas where burrows were prevalent and monitored during 2 consecutive seasons from May – August. Although the exit traps caught many sand flies and a few early instars *Locustae* spp., no *An. gambiae* s.l. were trapped exiting these burrows.

iii) **Caves** - The possibility that caves serve as aestivation shelters for *An.gambiae* s.l. was investigated since caves are recognized shelters for many species of mammals, birds and insects

(Happold 1987; Rosevar 1969). A number of small caves and rock crevices were identified and 5 were sampled nightly with CDC – battery powered suction traps. The traps were suspended inside the cave, between 1 and 1.5m from the opening and operated between 18:00 to 07:00. Each cave was sampled 4 successive nights. The morning following the last night of trapping, white drapes were placed within the cave and the resting fauna sampled using the PSC technique with additional drapes being placed over the entrance to prevent exit of insects. Additional caves were sampled using a combination of the back-mounted aspirator and PSC. CDC light traps failed to capture any *An. gambiae* s.l. although a number of gravid female *Culex quinquefasciatus* were caught. A back-mounted mechanical aspirator was used to sample the smaller rock holes. It also allowed rapid and thorough sampling from other sites of interest (e.g. cracks and crevices) which were not possible to sample by other means. A total of 20 such sites were sampled using this method 1 male *An.gambiae* s.l. was collected deep inside the largest cave.

**iv) Tree Holes, Wells & Rock Crevices.** Tree holes within an 800m radius from the center of both Doneguebougou and Banambani were sampled monthly by PSC. The purchase of a back - mounted aspirator enabled a period of intensive sampling to be performed at the end of the dry season during which a number of trees with previously inaccessible holes/hollows were also sampled. The equipment allowed rapid and thorough sampling from a number of other potential sites (e.g. wells, small rock holes/crevices.)

Table 1. Shows there were very few *An.gambiae* s.l. caught in these sites, although they appear to be suitable refuges for other species, particularly Culex. In the light of these data and year round sampling of tree holes in both villages, it would appear that the significance of tree holes and wells as potential aestivation sites is limited. As yet insufficient collections have been made from rock crevices throughout the dry season to confirm that this is also the case for these sites.

**Table 1.** *Summary of back-mounted aspirator catches from potential aestivation sites in the bush surrounding Donegubougou and Banambani villages. Number sampled = sum of both villages.*

| | Number sampled | *An. gambiae* s.l. | Other Anophelines | Other Culicidae |
|---|---|---|---|---|
| Tree holes | 379 | 5 male | 3 *An. rufipes* | >300 *Culex* spp. |
| Wells | 61 | 2 male | 1 *An. pharoensis*<br>4 *An. rufipes* | >280 *Culex* spp. |
| Rock holes/crevices | 75 | 3 male | 1 *An. rufipes* | 13 *Culex* spp. |

**v) Malaise Traps.** A number of Malaise traps were positioned around the village of Donegubougou in a attempt to sample potential aestivation sites which could not be sampled directly by active collecting or by exit traps. By intercepting mosquitoes flying on their way to breeding sites or the village we hoped to gain an indication of the presence of mosquitoes in certain target areas of the bush prior to the build up of the population in the village/larval sites. Ten Malaise traps were designed and built in Mali. The traps were 3m in length, 2 m high at each end (1.5m at the center) with a 0.75m overhang to channel insects flying into the mesh towards collecting vessels located at either end. The traps were designed to allow entry of insects into the collecting vessels from either side. Each collection vessel contained 300ml of Carnoy's fixative. Heavy-duty nylon mesh was used in the

construction of the traps, which were supported and held in situ by bamboo poles sunk into the ground. A ring of thorn branches encircled each trap which had been placed across an animal trail to prevent damage from cattle. Collecting vessels were checked daily (am and p.m.) for evaporation of fixative and catches emptied twice weekly at 3 & 4 day intervals. 15 male *An. gambiae* s.l. and 137 *Culex* sp. were captured.

**vi) Human bait catches.** Human bait catches (HBC) were carried out from April – July during the transition period from dry to rainy seasons with the intention of observing any build up in the population of *An.gambiae* s.l. in certain target areas, prior to their occurrence in the village. Two teams, each comprising two collectors, were employed at each site; the first team collecting from 1800 – 0000 h, the second from 0000 – 0600h. All mosquitoes landing on the exposed arms and legs of the collectors were sampled with mouth aspirators. Collections were separated into 2h time periods after which the collecting cups were changed. In each village site, the two collectors exchanged positions (inside/outside) every two hours. All mosquitoes caught were held in humid conditions to ensure their survival until the end of the experiment. Initial experiments yielded three female *An.gambiae* s.s. Subsequent catches were 57.6% *An.gambiae* s.s. and 42.41% *An.arabiensis*.

Failure to trap aestivating *An. gambiae* from the variety of sites investigated is not due to the application of inappropriate sampling techniques. In every case we have shown the efficacy of the sampling method by obtaining catches of other hematophagous insects. For example the capture of *Culex* mosquitoes and Phlebotomine sand flies from a termite mound indicates that these sites are certainly used by insects other than termites.

**Mark –Release-Recapture experiments** - A total of 9365 marked female *An.gambiae* s.l. were released in September 1999 - 4770 in Doneguebougou and 4595 in Banambani. Recaptures were carried out in all inhabited dwellings using mouth aspirators. Small-scale movement of mosquitoes between the two villages had been observed during the initial two weeks following the release but numbers of migrating mosquitoes went down sharply thereafter. From January 2000 no marked *An. gambiae* s.l. was recaptured.

**Larval sampling** - Weekly sampling of larvae offers the best opportunity to record the first occurrence of Bamako and Savanna chromosomal forms at the beginning of the rainy season before adults appear in the villages. Identification was performed by PCR.

**Automated temperature and humidity measurements** - Hobo® dataloggers were installed in 1999 and provided detailed information on the prevailing environmental conditions throughout the study period. The sampling frequency of each logger was set at 1 min with the hourly mean being recorded. By recording temperature and humidity from these sites we will gain invaluable information on what we feel are the two major environmental influences on aestivation. In addition to providing a possible correlation of environmental conditions with field catches, the data will also help to recreate "field conditions" for future laboratory studies in which we will elucidate the influence of photoperiod, temperature and humidity on aestivation. Examples of day and night means of both temperature and humidity are shown in Figs. 4-5.

**Figure 4.** Fluctuation in day and night temperatures (°C) and RH (%) recorded within human dwelling, Doneguebougou.

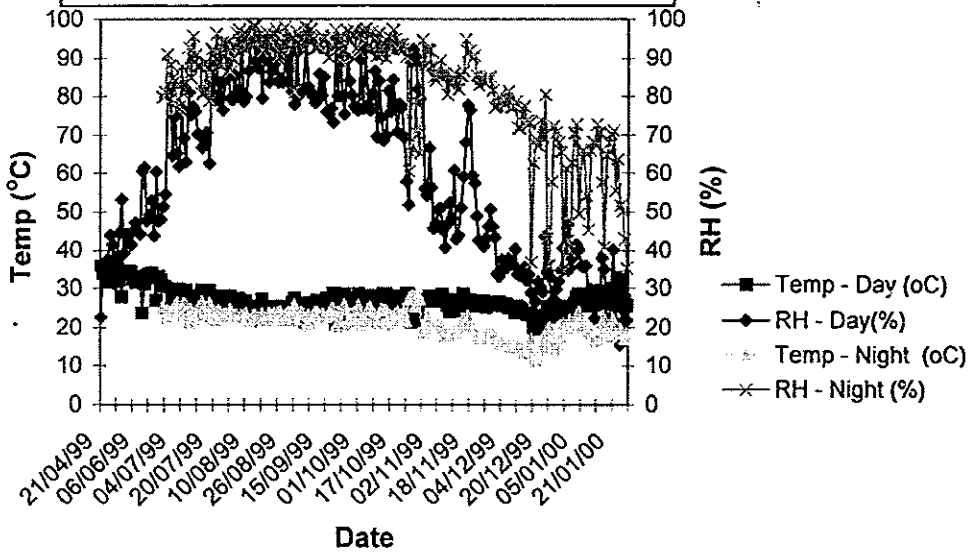
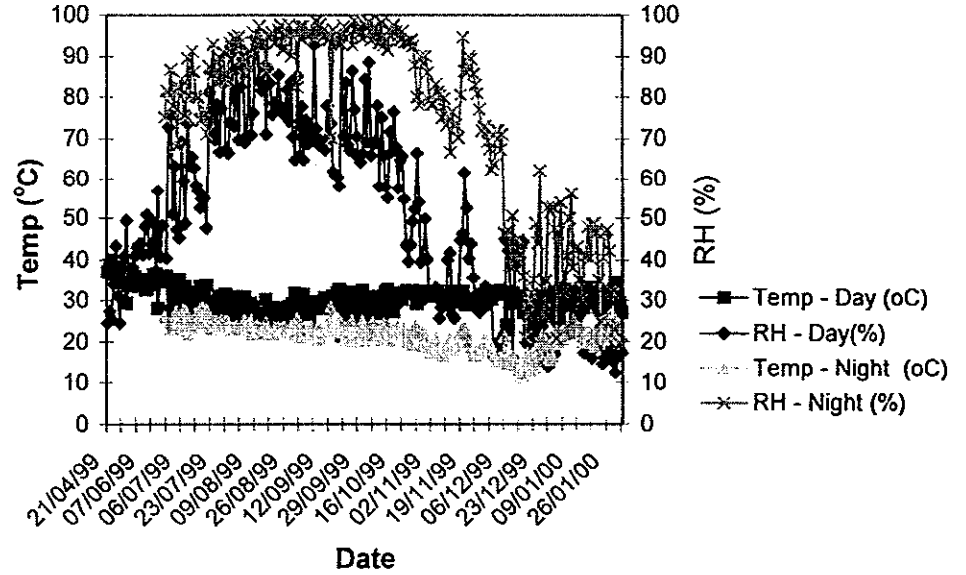



**Figure 5.** Fluctuation in day and night temperatures (°C) and RH (%) recorded within Mango grove, Doneguebougou.



**Aestivation studies in the laboratory** - Factors that may influence and/or control aestivation in the Mopti and Bamako forms of *An.gambiae* s.s. were investigated under laboratory conditions. Whilst current theories of the environmental factors influencing dormancy in insects are centered on temperature and photoperiod (Denlinger 1986), we initially investigated temperature and humidity. Meteorological data collected directly in the field since 1999 and that provided by the meteorological service of Mali demonstrated that these factors show the greatest, quantifiable change between rainy and dry seasons and, therefore, were deemed most likely to induce and maintain aestivation in *An.gambiae* s.l. (Holstein 1954)

**Colony establishment** - Colonies of Mopti and Bamako chromosomal forms were reared in the insectary from wild females collected in Moribabougou and N'gabacoro Droit. While Mopti proved relatively simple to maintain the Bamako colony failed to thrive. The existence of laboratory colonies of all three chromosomal forms of *An.gambiae* s.s. in Mali was a crucial step forward as it will allow us to initiate studies to induce aestivation in the laboratory. We are currently designing small C.T. chambers which will be used in Mali to assess the interactions of temperature, humidity and photoperiod on the biology of *An.gambiae* s.s.

**Standard Rearing Protocol: Blood & Sugar diet** - Males and females are maintained together until day 3-post emergence, males are removed and approximately 100 females are confined per per rearing container. Blood meals are provided on consecutive days 4 & 5. After the second blood meal mosquitoes are maintained on 4% sugar solution. Oviposition containers are provided on day 7 and egg counts are made the following morning. Larvae are maintained on commercial aquarium fish food, powdered and sprinkled on the water.

**Sugar diet** - Males and females remain together until day 3 -post emergence, males are removed while 100 females/container remain. Sugar pads are removed on the evening of day 3 but replaced (in lieu of blood) during days 4 & 5. Females are sacrificed (n=10) on day 9 and checked for abnormalities in ovaries and fat bodies.

**Blood diet** - Males and females remain together until day 3 -post emergence, males are removed and 100 females/container remain. Sugar pads are removed and blood meals are provided on days 4&5. Numbers of the females taking blood are noted. After the blood meal of day 5 the water pad is replaced. On the evening of day 7 females are allowed to oviposit and eggs are counted the following morning. A second oviposition on day 8 and egg count the following morning. Remaining females (n=10) are checked for the degree and stage of ovarian development on day 9.

Subsequent experiments were performed with slight modifications to the rearing regime.

| **Temp** / **Humidity** | **Standard = 27° C** | **High 35° C** |
|---|---|---|
| *Standard = 70%* | Baseline = rainy season | |
| **Low =15%** | Cool dry season | Hot dry season |

## 5.1.2. Laboratory studies - Israel.

A preliminary study in Israel using *An.gambiae* G3 strain had been conducted to identify possible technical problems in the protocols and to gain an idea of the effects of the dietary regimes on female physiology and longevity.

In the absence of a means mechanically controlling humidity in the controlled temperature (CT) chamber in Mali, saturated salt solutions were used to maintain humidity. A saturated NaCl solution was found to maintain humidity in the region of $80 \pm 2\%$ at the desired temperature (~27°). A saturated LiCl solution similarly maintained humidity within the holding chamber at $11.0 \pm 2\%$, regardless of temperature. We also investigated the effects of holding insects in enclosed chambers in close association with these salts. This was thought to be particularly important for LiCl, which is known to give off chlorine gas at temperatures in excess of 37°C. There were no adverse effects on the longevity of insects held over these solutions for a period of two weeks compared to females held in a CT chamber in which humidity was regulated to the same level.

**Respiratory Physiology.**

A major obstacle to studies of the respiratory physiology of adult mosquitoes is outbursts of locomotory activity which mask basal metabolic rates. Attempts to overcome this problem have employed surgical techniques (decapitation or removal of halteres) and have shown that respiration was reduced in haltereless females. Rates of oxygen consumption (VO2) of adult *Aedes aegypti* L. (male and female) typically increase for two days post emergence, lower by day 4 and show no subsequent correlation with age. Sugar feeding has been shown to increase $O_2$ consumption of both *Culex pipiens pipiens* and *Culex pipiens fatigans* to levels 2-3 times that of unfed individuals (Galun & Frankel, 1960).

Biochemical studies of nature of energy metabolism of adult mosquitoes have shown them to be characteristic of adult Diptera in that they utilize carbohydrate as an energy source for flight and lipid when at rest (Nayar & Van Handel, 1971). Glycogen and trehalose constitute the major carbohydrate reserves of female mosquitoes: glycogen being synthesized and stored in the fat body; trehalose is also synthesized in the fat body but is stored in the haemolymph. Lipids are stored principally in the form of trigycerides, in the fat body. The utilization of these energy reserves by starved, resting mosquitoes has been shown to proceed exponentially. Allowing access to sugar *ad lib* increases the deposition of both lipids and glycogen three times teneral levels (Briegel, 1990).

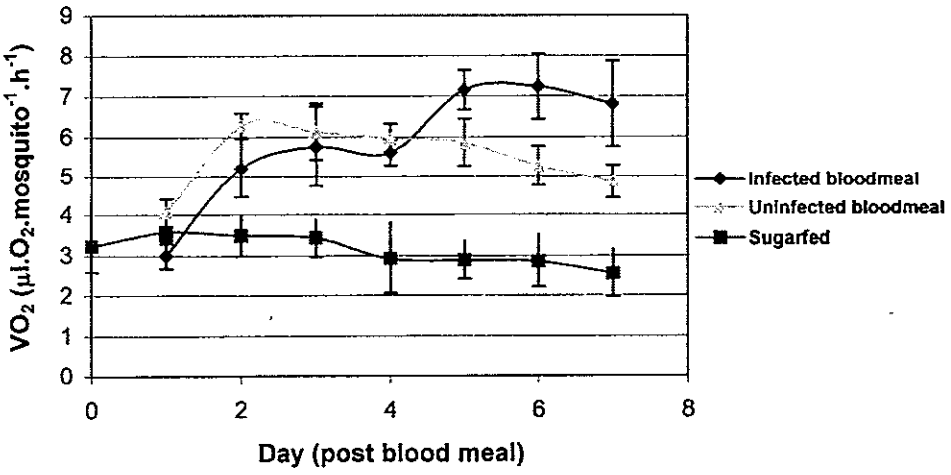
Blood feeding provides the female with a meal comprising mainly proteins and water. Blood-meal proteins are the major nutritional source for egg development, hence enzymatic activity within the midgut is predominantly proteolytic. Common to other enzymatic reactions, temperature greatly affects the rate of reaction (Billingsley & Hecker 1991). However, all the above studies have used midgut homogenates to elucidate the nature of protein digestion. We have approached this subject from a different perspective, using a non-invasive technique to correlate physiological changes and processes such as these with metabolic needs and expenditure.

We developed a new technique to measure both oxygen consumption and carbon dioxide production of groups of 10 mosquitoes. Insects, reared under pre-determined conditions, are introduced into a 2ml test chamber placed in a constant temperature water bath. The air stream (2ml/min) is humidified prior to entry into the test chamber by passing it through saturated salt solutions. Air exiting the test chamber is analyzed, first for $CO_2$ content by a Servomex Series 1400 IR $CO_2$ analyzer and then for $O_2$ content by an Ametex SA-3 $O_2$ analyzer. Insects are given an hour - long acclimatization period within the chamber and recording continues for a further hour. Sampling frequency is set to 0.2Hz and resting levels of $O_2$ consumption are calculated as the mean of at least 100 samples. Calibration of the analyzers before and after replicates allows accounts to be made in calculations of any drift in instrumentation. $O_2$ and $CO_2$ levels are calculated on the basis of the change in flow (at STP) of the air stream entering and exiting the test chamber.

This technique has been developed using *Aedes aegypti* as a model and the range of $O_2$ levels recorded are similar to those published previously using closed system. Respiratory rates of *An.gambiae* have also been measured, with initial results suggesting that there is a significant difference between the resting metabolic rates of the two species. Resting *An. gambiae* appears to consume much less oxygen per unit weight than resting *Ae. aegypti.*

Female *Ae.aegypti* maintained on a 10% sucrose diet showed an age-dependent decrease in oxygen consumption (Fig. 7.). When a similar aged cohort are fed chicken blood from an uninfected chicken $VO_2$ increases rapidly to peak 48 hours post meal. This metabolic peak coincides with peak peptidase activity and hence digestion of the blood meal. The high level of metabolism is maintained during ovarian development, highlighting the high energetic costs to the female, and is significantly lower only on day 6 (144 hours) post feeding.

**Figure 7.** Oxygen consumption rates ($VO_2 \pm SD$) of female *Ae.aegypti* fed *P.gallinaceum* infected and uninfected chicken blood.

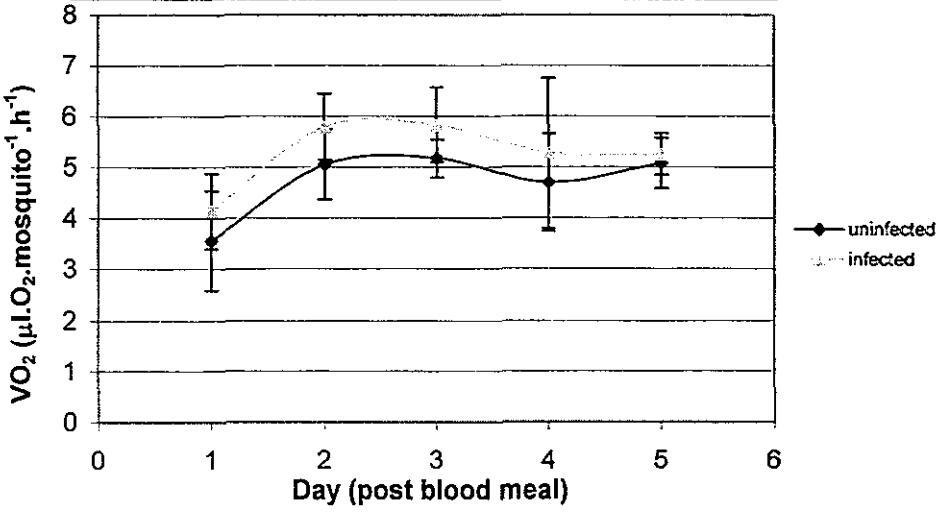



Feeding females on chickens with 30-35% *P.gallinaceum* parasitaemia produces a similar $VO_2$ curve for the first 3 days post blood meal, however, on day 5 post blood meal there is a further significant increase in $VO_2$ compared to the earlier digestive activity peak (Fig.8). Although this

increased metabolic activity diminishes with time, it remains higher than that in similar aged mosquitoes fed uninfected chicken blood.

In order to elucidate whether this pattern of increase in metabolic activity was in some way parasite induced, two groups of chicken blood (uninfected and *P.gallinaceum* infected) were subjected to freeze-thaw cycling to kill parasites. Blood smears revealed that no viable parasites remained in the sample of infected chicken blood. Female mosquitoes were then presented this blood via a membrane feeder. Oxygen consumption curves of those females that fed to repletion were subsequently produced (Fig 8.). There was no significant difference between the two profiles produced, suggesting that the increase in metabolic activity recorded with infected blood was parasite induced.

**Figure 8.** Oxygen consumption rates ($VO_2 \pm SD$) of female *Ae.aegypti* membrane- fed Blood that was subjected to "freeze-thaw" cycling. Yellow line=i nfected (*P.gallinacaeum*) blue= uninfected chicken blood .




## 6. Impact, relevance and Technology transfer

Dr A Bhasin, the Postdoctoral fellow who was sponsored by this program, made extended visits to Mali. In Mali, Dr Bhasin worked closely with Adama Dao, his Malian colleague, on the technical and practical aspects of the study. Field trips were joint ventures of both the Israeli and Malian investigators and were made twice monthly during the periods Dr Bhasins was in Mali. Dr Warburg, the Israeli PI spent 3X2 weeks in Mali. He participates in field work, assessed the progress of the sampling regime and conducted discussions with Y. Toure and the other Malian scientists. He also delivered 2 scientific seminars on topics related to malaria transmission.

Adama Dao made 2 visits to Israel during which he was trained into disparate fields:
With significant progress made in Mali towards completing the identification of mosquito samples, it was imperative to collate the data and design a working database that could be used by both teams. This database was successfully set up by A. Dao and A. Bhasin and facilitated the transfer of data between the two teams. The data base is updated regularly by the Malian Scientists and is still being used in Mali.
During his stays A. Dao was also made familiar with the technologies involved in measuring metabolic rates of individual mosquitoes in the laboratory. Upon his return, A. Dao constructed a functional experimental setup for determining respiration rates in wild-caught mosquito's wit ha view to identifying those that may have been in aestivation. Unfortunately, with the end of the current project, Mr. Dao left for a PhD of the program outside Mali, and Prof Toure assumed a position in WHOM. Thus, those parts of the program that were not completed are currently pursued by persons who were not specifically trained for them..

**7/8. Project Productivity / Output**

Despite unforeseen difficulties, many interesting findings were made. Two manuscripts acknowledging CDR support have been submitted for publication. The first one summarizes all the data on mosquitoes trapped in the dry season, locations, gonotrophic and ovarian stages and ecophenotype. Despite the relatively small number of *An. gambiae*, found, compilation of project – derived data with data found but not analyzed in MRTCBKO we have the most comprehensive information base describing this important ecological phenomenon.

In addition, the open-flow respirometry of individual mosquitoes with or with out malaria infections is a worthy achievement for publication in a specialized entomological journal.

**9. Future work**

Due to the fact that the Malian team leaders, Prof Y.T. Toure and Mr. A. Dao are currently out of the country and because funding by CDR has terminated, we are currently not proceeding with the project as we would have liked. We are however, confident that once these two scientists return, we shall continue the project and determine how and where *An. gambiae* aestivating mosquitoes are located during the dry seasons of Sub-Saharan regions.

**10. Literature**

Ar A. & Piontkewitz Y. 1992. *Respir physiol,* 87: 407-418.

Arieli, R. (1979). *Comp Biochem Physiol*, **63A**:569-575.

Billingsley PF, Hecker H. 1991.. *J Med Entomol.* **28**:865-71.

Briegel H. 1990.. *J Med Entomol* **27**:839-850.

Denlinger D.L. (1986). *Annu Rev Entomol*, 31: 239-264.

Doumbo O, Traore SF, Sow Y, Dembele M Soula G, Coulibaly A, Dolo A, Sangare O,

Koita O, Pichard E & Toure YT. 1991. *Bull. Soc. Path. Ex.* 84: 761-774.

Galun R. & Fraenkel G. 1961. *J Insect Physiol*, **7**: 161-176.

Happold, D.C.D. (1987). Clarendon Press, Oxford.

Holstein M.H. (1954).. WHO monogr ser 172 pp.

Nayar JK & Van Handel E (1971). *J Insect Physiol.* Dec;**17**:2475-2479.

Omer, S.M. & Cloudsley-Thompson, J.L. (1968). *Bull WHO*, **42**:319 330.

Rosevar, D.R. (1969). *Brit Mus (Nat Hist)*, Lond.

Sangare D., Traore S.F, Bagayogo M., Bouare M., Dolo G., Sow M.Y., Toure A., Sogoba

N., Dao A., Petrarca V., Sakai R., Toure Y.T. 1994. *Am J Trop Med Hyg*, **51**: 476.

Storey, K.B. & Storey K.M. (1990). *Quarterly Rev Biol,* 65: 145-174.

Taylor C.E., Toure Y.T., Coluzzi M. & Petrarca P., 1993. *Med Vet Entomol*, **7**: 351-357.

Toure Y.T. Petrarca V., Coluzzi M. 1983. *Parassitologia* 25: 367-370.

Washino R.K. (1969). *Ann Entomol Soc Am*, **63**: 210-216.

Williams D.D. & Rausch (1973). *Comp Biochem Physiol*, **44A**: 1227-1235.

Wilson, K.J. & Kilgore, D.L. (1978). *J Theor Biol*, **71**:73-101.